Association between Physical Activity and Serum Bilirubin Levels and Its Potential Modulating Effect in Trained and Untrained Adult Males

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Abstract: Why studies over the last two decades had shown low levels of serum bilirubin to be associated with high risk for varieties of systemic diseases in human, we propose that one potential modifiable behavior to increase bilirubin levels is physical activity. The purpose of this study was to examine the association between physical activity and serum bilirubin levels and its potential modulating effect among trained and untrained adult males. Employing purposeful sampling technique, 20 trained and 20 untrained adult males were recruited for this study following inclusion and exclusion criteria. The university institutional review board (ABUTH/HREC/TRG/36) gave approval for all procedures in accordance with the Declaration of Helsinki. Blood samples were taken to measured serum total bilirubin and leukocyte counts respectively from all subjects at rest. The VO2Max was estimated from a standard regression equation. The independent students-t-test was used to compare values between the two groups. Linear regression analysis was also used for prediction of any association. The level of significance was set at P< 0.05. Our result showed that VO2 max exhibited significant differences between the trained and untrained, leukocyte counts in the untrained group (140.10±1.65 x 50 mm3) was significantly (p< 0.05) higher compared to the trained group (134.50±2.46 x 50 mm3). On the other hand, serum total bilirubin in the trained group (11.35±2.6 mmol/l) was significantly (p < 0.05) higher compared to the untrained group (4.80±1.6 mmol/l). The VO2Max, correlated positively with serum total bilirubin (p < 0.0001), in both the untrained group (R2= 0.002, +0.045) and in the trained group (R2= 0.088, +0.297) respectively. In addition, Leukocyte counts correlated negatively with serum total bilirubin (p < 0.0001), in the untrained group (R2= 0.162, -0.403) and correlated positively in the trained group (R2= 0.032, +0.178; p < 0.0001). With the exciting results revealed, physical activity was positively associated with bilirubin among the trained males in an increasing trend. This means that an increased physical activity might increase hemoxyginase-1 activity which is the enzyme responsible for the conversion of biliverdin to bilirubin. In addition, leukocyte counts in the untrained was higher compared to the trained which means the potential modulating effect of the bilirubin might be on inflammation process. This study is novel, as, to our knowledge, no studies to date have examined this association. This finding has implications for sports physicians, diagnosis or applied exercise physiology.

Keywords: trained, untrained, physical activity, bilirubin, leukocyte
INTRODUCTION
Stress is usually considered as an aversive stimulus capable of altering physiological homeostasis whose intensity has detrimental effects (23). However, frequent and regular exercise stress has been shown to provide various health benefits (12). But on the other hand, exercise causes stress on human organism (1) inducing different physiological and metabolic effects. These effects, however, might depend on the severity, duration and frequency of the exercise as well as physical and physiological condition of the subjects (16). In order to achieve favorable physiological changes from regular exercises, loading elements such as severity and frequency of exercise should be well-defined (27). In addition, adequate monitoring of the functional status is necessary to ensure the quality of recovery and hence further successful participation in professional athletic activities (18). Monitoring of recovery is usually complicated due the absence of simple and valuable indicators. The wide choice of metabolic markers in sports medicine was recently systematized by (4). Why it is well documented that, cholesterol, blood pressure, and glycemic regulation, pivot an important role in physical and mental health (17), serum bilirubin has been considered to be a new biomarker for varieties of chronic diseases (7). Researches over the last two decades had shown low levels of serum bilirubin to be associated with high risk for cardiovascular disease (9), metabolic syndrome (11), type 2 diabetes (13), stroke severity (29), certain cancers (31), autoimmune disease (30), and psychiatric disorders (22). Bilirubin has been shown to modulate the risk of these diseases by reducing lipid peroxidation or mitigating inflammation (10, 28). Looking at the antioxidant properties of bilirubin (28), cost-effective strategies to increase bilirubin levels are required. We propose that one potential modifiable behavior to increase bilirubin levels is physical activity; because regular participation in physical activity has been shown to increase antioxidant enzyme and coenzyme activities (2). However, to increase our knowledge base of the potential association between physical activity and serum bilirubin level in trained; the purpose of this study was to examine the association between physical activity and serum bilirubin levels and its potential modulating effect among trained and untrained adult males.

MATERIALS AND METHODS
Subjects
This cross-sectional design study involved twenty trained (age: 20.60±0.54years; weight: 60.25±1.3 kg; height: 1.76±0.0m) and twenty untrained (age: 20.00±0.48years; weight: 58.60±1.0kg; height: 1.70±0.0 m); adult male volunteers, via purposeful sampling technique. All trained subjects were members of soccer clubs and had been training for the past four years or longer, at least 4 days per week, with training sessions lasting 1–1.5 hour. The twenty untrained males had not been playing any sports regularly. All subjects who had no history of diseases, not using steroids or other banned substances, non-smokers, and no history of bleeding disorders, were included to participate in this study. The university institutional review board (ABUTH/HREC/TRG/36) gave approval for all procedures in accordance with the Declaration of Helsinki. Subjects were required to report to our research laboratory to read and sign a medical questionnaire and an informed consent in accordance with the American College of Sports Medicine (3) guidelines. Subjects were fully informed about the experimental procedures, risks and protocol, knowing that
they can withdraw voluntarily at any given time of the experiment.

PROCEDURES

Determination of physical activity
This study employed an objective measure of physical activity (i.e., VO\(_2\)max), determined by the product of the arteriovenous oxygen difference and cardiac output which is not merely an index of physical fitness, but also reflects the function of the cardiopulmonary system and the prevalence of risk factors for atherosclerosis (14). All subjects were assessed for VO\(_2\)max as described by (15). The estimation of the VO\(_2\)max with this test requires a score from a simple exercise history questionnaire in addition to age, height, weight, and gender. No exercise was performed but a measure of past exercise was determined by the questionnaire. The VO\(_2\)max was then computed using the formula:
\[
\text{VO}_2\text{max} \ (\text{ml.kg}^{-1}.\text{min}^{-1}) = 56.363 + (1.921 \times \text{PA-R}) - (0.381 \times \text{AGE}) - (0.754 \times \text{BMI}) + (10.987 \times \text{Gender})
\]

Where: Male = 1, Female = 0

BMI = Weight in kg / Height\(^2\) in meters

PA-R = Score on the physical activity questionnaire

Results obtained were rated using a standard VO\(_2\)max table.

BLOOD SAMPLING
Venous blood samples were collected into plain and heparinized evacuated tubes from a forearm vein with minimal stasis after approximately 10 min of rest in a sitting position between 8 and 9 am, after an overnight fast and at least 24 hours from the last workout.

**Determination of White blood cell count**
The WBC count was done using the Turks method as described by (6).

**Determination of total serum bilirubin**
Total serum bilirubin and was determined according to the method described by (24) and was measured with a laboratory automat (Selectra XL Vital Scientific, Spankeren, NL) as adopted by (21).

**STATISTICAL ANALYSIS**
The data generated were expressed as mean ± standard error of mean. For statistical analysis, SPSS software (version 20.0) was used; the independent students-t-test was used to compare values between the two groups. Linear regression analysis was also used for prediction of any association. A comparison was considered statistically significant if the P value was less than 0.05 i.e. \(p < 0.05\).

**RESULTS**
The mean physical characteristics of the study population were presented in Table 1. There was no significant difference (\(p > 0.05\)) in age and body weight between the untrained group and the trained group. However, BMI, heart rate, diastolic blood pressure and VO\(_2\) max exhibited significant differences between the untrained and trained groups, leukocyte counts in the untrained group (140.10±1.65 x 50 mm\(^3\)) was significantly (\(p < 0.05\)) higher compared to the trained group (134.50±2.46 x 50 mm\(^3\)) (Table 1). On the other hand, serum total bilirubin in the trained group (11.35±2.6 mmol/l) was significantly (\(p < 0.05\)) higher compared to the untrained group (4.80±1.6 mmol/l). The associations of VO\(_2\)Max, with Total Bilirubin, for both groups were determined by univariate regression analysis. VO\(_2\)Max, correlated
positively with serum total bilirubin ($p < 0.0001$), both in the untrained group ($R^2 = 0.002, +0.045$) and in the trained group ($R^2 = 0.088, +0.297$) (Table 2, Fig. 1, Fig. 2) respectively. In addition, the associations of leukocyte counts, with Total Bilirubin, for both groups were also determined by univariate regression analysis. Leukocyte counts correlated negatively with serum total bilirubin ($p < 0.0001$), in the untrained group ($R^2 = 0.162, -0.403$) and correlated positively in the trained group ($R^2 = 0.032, +0.178; p < 0.0001$) (Table 3 Fig. 3, Fig.4).

**Table 1. Differences in risk factors in the untrained and trained groups**

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>UNTRAINED</th>
<th>TRAINED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(yrs.)</td>
<td>20.06±0.48</td>
<td>20.62±0.54</td>
</tr>
<tr>
<td>BMI(Kg/m²)</td>
<td>20.20±0.31</td>
<td>19.25±0.29*</td>
</tr>
<tr>
<td>Heart Rate(Beat/Min)</td>
<td>80.40±3.64</td>
<td>65.45±2.17*</td>
</tr>
<tr>
<td>Systolic Blood Pressure(mmHg)</td>
<td>128.0±2.40</td>
<td>128.8±2.10</td>
</tr>
<tr>
<td>Diastolic Blood pressure(mmHg)</td>
<td>69.05±1.89</td>
<td>74.15±1.80*</td>
</tr>
<tr>
<td>VO₂ max (mL/kg/min)</td>
<td>50.30±1.50</td>
<td>58.40±1.70*</td>
</tr>
<tr>
<td>Leukocyte counts(×10⁹/L)</td>
<td>14.10±1.65</td>
<td>13.45±2.45*</td>
</tr>
<tr>
<td>Total serum bilirubin(μmol/L)</td>
<td>4.50±1.6</td>
<td>11.35±2.6*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM.

BMI, body mass index, VO₂ max, maximum oxygen uptake

* indicate significant difference at $p < 0.05$ when compared with untrained group.

**Table 2. Association between VO₂ Max and Total Bilirubin in untrained and trained adult males calculated by simple regression analysis**

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Total Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untrained</td>
</tr>
<tr>
<td>VO₂ max (mL/kg/min)</td>
<td>beta</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Data are expressed as the coefficient of correlation.

VO₂ max, maximum oxygen uptake
DISCUSSIONS
The findings of our study was that the VO\textsubscript{2} max and total bilirubin were higher in the trained group compared to the untrained group. On the other hand, leukocyte counts were higher in the untrained group compared to the trained group. In addition, our univariate regression analysis showed that total bilirubin was correlated to VO\textsubscript{2} max and Leukocyte counts respectively. Total bilirubin correlated positively with VO\textsubscript{2} max (p < 0.05) in both the trained and the untrained groups. The result from the present study disagreed with the findings of the previous authors. In 2008, (8) reported no association between cycling training and bilirubin levels in lean or obese adults. Most recently, in 2013, (26) reported no association between self-reported physical activity and bilirubin levels. In addition, (19), in his study, the comparison of biochemical blood levels of athletes and sedentary, reported insignificant decreased in the serum total bilirubin concentrations in the athletes compared with sedentary. The differences between the findings of these previous authors and the present study might be due to the objective measure of physical activity employed and the mode of training. It was difficult to explain why physical activity was positively associated with bilirubin in an increasing manner among the trained males. A possible explanation, however, is that, increased physical activity could increase hemoxygenase-1 activity (17), which is the enzyme responsible for the conversion of biliverdin to bilirubin. In addition to increase in HO-1 activity, other potential mechanisms include physical activity-induced intravascular hemolysis (5) following training adaptation. Speculatively, some mode of exercise may not be sufficient enough to increase the activity of the HO-1 system or induce hemolysis, which could be the reason for the differences observed between this study and those of the previous authors. Furthermore, several studies had shown that, low levels of serum bilirubin were associated with increased risk cardiovascular disease (9), metabolic syndrome (11), type 2 diabetes (13), stroke severity (29), certain cancers (31),
autoimmune disease (30), and psychiatric disorders (22). However, the high levels of serum bilirubin in the trained group (footballers) from the present study, might likely explain the health benefit the training confer to them. Since bilirubin is conceived to modulate the risk of these diseases by reducing lipid peroxidation or mitigating inflammation process (10, 28), the present study, also show that total bilirubin correlated negatively with leukocyte count in the untrained group and positively in the trained group. This result, also proposed that, the potential modulating effect of the bilirubin might be on inflammation process. This study is novel, as, to our knowledge, no studies to date have examined the association between VO$_2$ max and bilirubin in trained and untrained adult males. Limitations of the present study include the cross-sectional design, rendering causal inferences not possible. Additionally, and although total bilirubin are linked with cardiovascular risk (20), it was not possible to assess other components of bilirubin such as free, conjugated or unconjugated bilirubin. Despite these limitations, major strengths of this study include an investigation of this understudied topic, using an objective measure of physical activity, and employing a representative sample of trained and untrained adult males.

CONCLUSION

The present cross-sectional study demonstrated that, physical activity was positively associated with bilirubin level among the trained males in an increasing trend. This means that an increased physical activity might increase hemooxyginase-1 activity which is the enzyme responsible for the conversion of biliverdin to bilirubin. In addition, leukocyte counts in the untrained was higher compared to the trained which means the potential modulating effect of the bilirubin might be on inflammation process. This study is novel, as, to our knowledge, no studies to date have examined this association. This finding has implications for sports physicians, diagnosis or applied exercise physiology. Further researches are needed, with much focuses on the mechanism explaining the association between physical activity and bilirubin in trained.

CONFLICT OF INTERESTS

The authors have no conflict of interests to disclose in this study.

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REFERENCES